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Effect of Turbidity on Chlorination Efficiency and Bacterial Persistence in Drinking Water†

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To define interrelationships between elevated turbidities and the efficiency of chlorination in drinking water, experiments were performed to measure bacterial survival, chlorine demand, and interference with microbiological determinations. Experiments were conducted on the surface water supplies for communities which practice chlorination as the only treatment. Therefore, the conclusions of this study apply only to such systems. Results indicated that disinfection efficiency ($\log_{10}$ of the decrease in coliform numbers) was negatively correlated with turbidity and was influenced by season, chlorine demand of the samples, and the initial coliform level. Total organic carbon was found to be associated with turbidity and was shown to interfere with maintenance of a free chlorine residual by creating a chlorine demand. Interference with coliform detection in turbid waters could be demonstrated by the recovery of typical coliforms from apparently negative filters. The incidence of coliform masking in the membrane filter technique was found to increase as the turbidity of the chlorinated samples increased. The magnitude of coliform masking in the membrane filter technique increased from <1 coliform per 100 ml in water samples of <5 nephelometric turbidity units to >1 coliform per 100 ml in water samples of >5 nephelometric turbidity units. Statistical models were developed to predict the impact of turbidity on drinking water quality. The results justify maximum contaminant levels for turbidity in water entering a distribution system as stated in the National Primary Drinking Water Regulations of the Safe Drinking Water Act.

The National Interim Primary Drinking Water Regulations promulgated on 24 December 1975 in accordance with the Safe Drinking Water Act (Public Law 93-523) established a maximum contaminant level for turbidity in drinking water (24). A turbidity of 1 nephelometric turbidity unit (NTU) was recommended, and up to 5 NTU were allowed if the supplier could demonstrate that turbidity did not interfere with disinfection, prevent the maintenance of effective disinfectant in the distribution system, or interfere with microbiological determinations. The regulations also required that daily turbidity samples be taken from representative entry point(s) to the water distribution system and that measurements be made with a nephelometer, and if a turbidity over 1 NTU is obtained, the measurement should be confirmed by sampling the water, preferably within 1 h.

Turbidity in water is caused by the presence of suspended matter such as clay, silt, organic and inorganic matter, plankton, and other microscopic organisms (1, 12). Turbidity is an expression of the optical property of water that causes light to be scattered and is measured by determining the degree of light scattering by particulates present in the samples.

Previous research concerning the effects of turbidity on drinking water potability have associated coliforms with nematodes, crustaceans, iron rust, and plankton inside the distribution system (2, 3, 20, 23). Water that was experimentally contaminated with feces containing infectious hepatitis virus and then chlorinated produced illness in volunteers, whereas a similar water sample that was coagulated and filtered to remove turbidity and then chlorinated produced no illness (17). Unpleasant odors and tastes have been associated with high turbidities (15). Turbidity can carry nutrients to support microbial growth in the distribution system (8, 9); the ensuing high standard plate counts can mask the detection of coliforms (8, 9, 11, 13, 19, 25). Despite these observations, no experimental evidence exists as to the impact of turbidity at entry points to a distribution system in which no treatment other than chlorination is practiced. Since many small communities do not practice full water treatment, the purpose of this research

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was to evaluate the quantitative impact that turbidity has on disinfection efficiency, maintenance of a chlorine residual, and microbiological determinations and to relate these findings to the 1- and 5-NTU maximum contaminant levels.

MATERIALS AND METHODS

Sample area. Drinking water samples were collected from a community which used chlorinated surface water as the drinking water supply (5). Untreated surface water samples were collected from six watersheds located in western Oregon. Four of the watersheds, Schooner Creek, Bear Creek, Oak Creek, and Rock Creek, receive no industrial or domestic wastes. The other two watersheds, Mary's River and Willamette River, receive domestic effluent and agricultural runoff. In some of the watersheds, logging operations have left upstream slopes reduced in vegetation, making the hillsides subject to erosion.

Collection of samples and microbiological techniques. Turbid raw water was collected in the area of the municipal intake in sterile polypropylene carboys by two different methods. One method was to collect water during or after periods of precipitation, when the surface water was turbid. The other method was utilized during dry periods. Sediment was collected from the stream bottom (top 5 to 10 mm) and added to the surface water to achieve various turbidity levels. The chlorine demand of the samples was determined by the *Standard Methods* procedure (1). A stock solution of chlorine was prepared daily from calcium hypochlorite (Fisher Scientific Co., Pittsbrugh, Pa.) and standardized by amperometric titration (Fischer and Porter titrator). The pH values of the samples after chlorine addition were all within 0.2 U of neutrality. Samples were incubated in the dark for 1 h at 10°C. The incubation temperature was comparable to the mean yearly temperature of the surface water supplies. At the end of this time interval, free and total chlorine residuals were determined by amperometric titration. Samples retained for microbiological analysis were dechlorinated, using sodium thiosulfate (Mallinckrodt, Inc., St. Louis, Mo.). The turbidity of the samples was measured with a Hach model 2100A turbidimeter. Formazin turbidity standards were prepared weekly in accordance with *Standard Methods.* (1). Nitrate, orthophosphate, suspended solids, and total organic carbon content of the water were all determined in accordance with *Standard Methods* (1). Total coliforms were enumerated by the membrane filter (MF) technique with an added resuscitation step by using lauryl tryptose broth (Difco Laboratories, Detroit, Mich.)-saturated pads, m Endo Agar LES (Difco), and Gelman (GN-6; 0.45 μm pore size) membrane filters (1). Typical coliform colonies were verified for gas production in lauryl tryptose broth (Difco) and brilliant green lactose bile broth (Difco). Chlorinated turbid water samples were blended for 1 min in 15-s intervals at 20,000 rpm in a Waring 700 commercial blendor. Zwittergent 3-12 (Calbiochem, La Jolla, Calif.) was added to water samples before blending (10^{-6} M, final concentration) to help disperse bacteria. Standard plate count bacteria were enumerated on blended and unblended samples by the MF technique (22).

Coliform masking. Interference with coliform detection in the MF technique was demonstrated by placing filters with no typical green sheen colonies into tubes of lauryl tryptose broth and processing as shown in Fig. 1. The multiple-inoculation and isolated-colony procedures were performed as previously described (5). Coliforms were identified by methods previously described (4, 13). A most-probable-number (MPN) index was generated by filtering triplicate volumes of three 10-fold dilutions and processing the filters in the manner described above. The magnitude of masking was determined by subtracting the original MF verified count from the MPN index. We recognize the potential dangers of using these different techniques to measure the efficiency of coliform recovery. However, a side-by-side comparison was essential in order to estimate the differential effect that turbidity has on the MF technique.

Statistical analyses. Regression models were developed using an established computer program and accepted techniques (18).

Scanning electron photomicrographs. Turbid samples were filtered through 0.4-μm polycarbonate filters (Nuclepore Corp., Pleasanton, Calif.) and fixed with cold 3% glutaraldehyde in Sorensen's phosphate buffer (pH 7.0). Filters were then postfixed in 1% osmium tetroxide, dehydrated in graded ethanol, and critical-point dried by using CO_2. Filters were mounted on sample stubs with copper tape, sputter coated (Hummer V-Technics, Alexandria, Va.) with 30 nm of gold-palladium (60:40), and examined with a JEOL 100 CX-ASID electron microscope (Japanese Electron Optics Laboratory, Boston, Mass.) at 40 kV.

RESULTS

The influence of turbidity on drinking water quality was examined in six watersheds where turbidities at entry points to distribution lines ranged from 0.2 to 15.0 NTU (geometric mean of 2.4 NTU). Coliform densities in raw water ranged from 11 to 500 coliforms per 100 ml (geometric mean of 88 coliforms per 100 ml) and were reduced 10- to 1,000-fold (geometric mean of 63-fold) by chlorination.

The relationship between turbidity and chlorine disinfection efficiency was determined by measuring the decrease in numbers of coliforms at different turbidity values and different concentrations of added chlorine (Fig. 2). The results indicated that coliforms in high-turbidity water (13 NTU) were reduced to only 20% of the initial count, whereas coliforms in low-turbidity water (1.5 NTU) were undetectable even when large volumes were sampled (1,000-fold or greater decrease). Disinfection efficiency was measured as $\log_{10}$ of the decrease in coliform numbers and was found to be negatively correlated with turbidity ($r = -0.777$; $P < 0.01$).

Turbid water samples were examined in an attempt to determine factors which aid bacteria in surviving exposure to chlorine. Scanning electron photomicrographs (Fig. 3) demonstrated that some bacteria were either embedded in turbidity particles or appeared to be coated with amorphous material or both. Blending of chlorinated turbid water increased the number of standard plate count bacteria as much as five times, indicating the physical separation of cells attached to common particles.

The chlorine demand (CLDMD) of the surface water supplies was found to be positively correlated with both the turbidity and total organic carbon (TOC) content of the water (Fig. 4 and 5), but not with other chemical parameters. The association of CLDMD with turbidity and TOC could be described by the equations: CLDMD = 0.040 + 0.086 NTU (r = 0.85; $P <$ 0.01) and CLDMD = 1.36 + 0.525 TOC (r = 0.95; $P <$ 0.01), where TOC is measured in milligrams per liter. To explain the association of CLDMD with TOC and turbidity, turbid samples were filtered by using prewashed filters

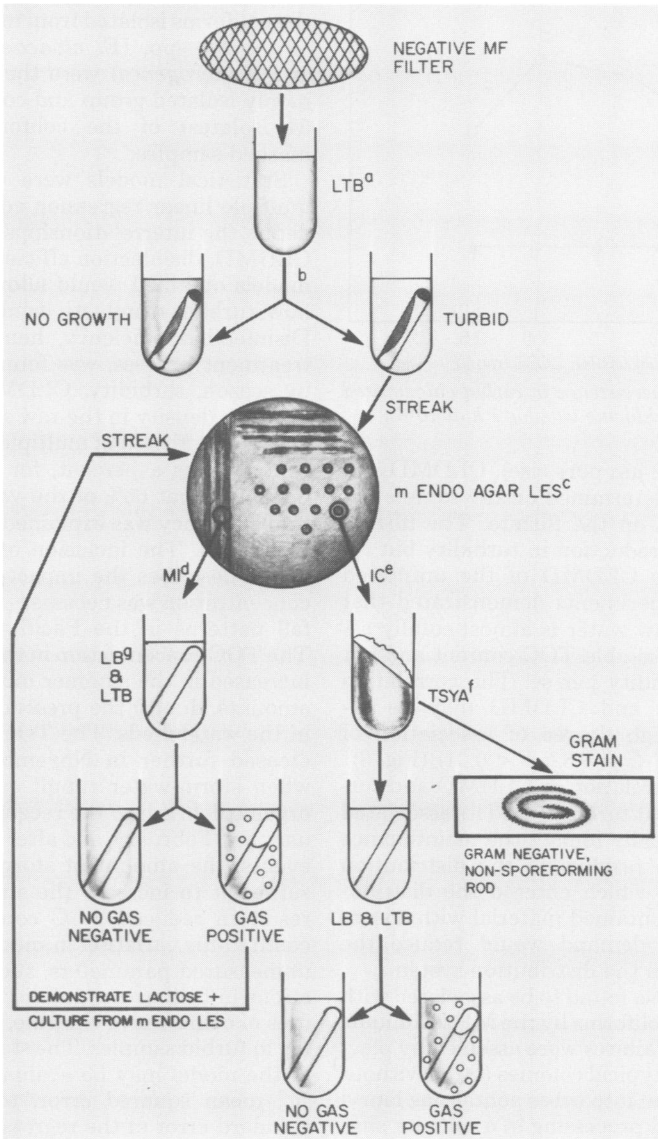

FIG. 1. *Flow scheme for demonstrating coliform masking in the MF technique. (a) Lauryl tryptose broth; (b) tubes examined after 48 h at 35°C; (c) m Endo agar LES; (d) inoculation of multiple colonies from heavy growth portion of the plate; (e) inoculation of an isolated colony; (f) tryptic soy-yeast extract agar; (g) lactose broth.*

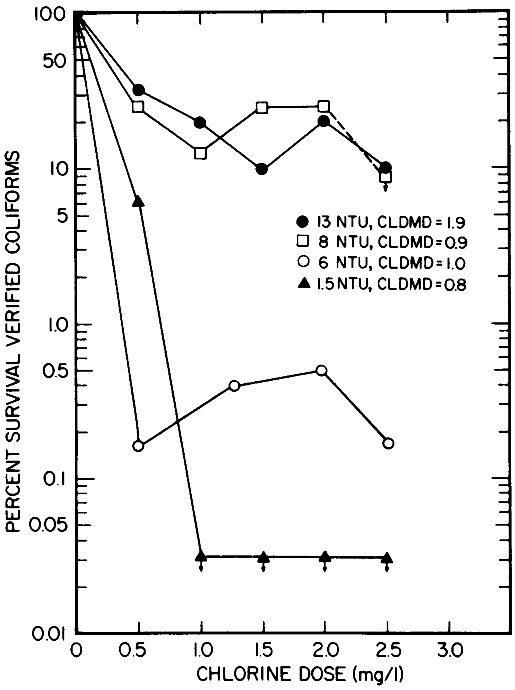

FIG. 2. *Coliform persistence in turbid chlorinated water. Exposure to chlorine was for 1 h at 10°C.*

(Millipore GS; 0.22-μm pore size). CLDMD and TOC levels were determined on the sample before filtration and on the filtrate. The filtrate exhibited a 99.9% reduction in turbidity but retained 90% of the CLDMD of the unfiltered sample. These experiments demonstrated that the CLDMD of raw water is almost totally associated with the soluble TOC content and not directly with turbidity per se. The correlation between turbidity and CLDMD may be explained by the high degree of association of turbidity and TOC ($r = 0.82$; $P < 0.01$) (Fig. 6).

The positive correlation of CLDMD and turbidity indicated that turbidity and its associated TOC could potentially impact the maintenance of a free chlorine residual in the distribution system. Turbidity which entered the distribution system and contained material with an unsatisfied chlorine demand would reduce the chlorine residual in the distribution system.

Turbidity was also found to be associated with failures to detect coliforms by the MF technique. The MF coliform failures were assessed by placing filters without typical colonies (often without any visible colonies) into tubes containing lauryl tryptose broth and processing in a manner similar to the modified MPN technique (5). The incidence of false-negative results or coliform masking in the MF technique increased as turbidity increased (Fig. 7). At turbidities of ≤1 NTU, 17% of the filters which were initially free of visible typical colonies were found to be coliform positive. The frequency of false-negative results increased to 45% at 5 NTU and to over 80% at turbidities in excess of 10 NTU. The magnitude of coliform masking was less than 1 coliform per 100 ml for 83% (10/12) of the samples having turbidities below 5 NTU, whereas the magnitude of masking was greater than 1 coliform per 100 ml for 75% (6/8) of the samples having turbidities higher than 5 NTU. *Citrobacter freundii* comprised 60% (32 of 54 isolates) of the coliforms isolated from masked samples. *Enterobacter* spp. (*E. cloacae*, *E. agglomerans*, and *E. aerogenes*) were the second most commonly isolated group and comprised 30% (16 of 54 isolates) of the coliforms isolated from masked samples.

Statistical models were developed through multiple linear regression computer analysis to define the interrelationships between turbidity, CLDMD, disinfection efficiency, and TOC. The models obtained would allow predictions as to how turbidity impacts drinking water quality. Disinfection efficiency, hence, efficacy of the treatment process, was found to be influenced by season, turbidity, CLDMD, and the initial coliform density in the raw source water (Table 1). The coefficient of multiple determination, R^2, expressed as a percent, for model 3 (Table 1) indicated that 66% of the variation in disinfection efficiency was explained by the variables in the model. The inclusion of a numerical term which describes the impact of season on TOC concentration was necessary because of the rainfall patterns in the Pacific Northwest region. The TOC concentration in the streams gradually increased in the summer months of low rainfall amounts, due to the presence of organic debris in the watersheds. The TOC concentrations increased further in November and December, when storm-water runoff carried accumulated organic debris into the receiving stream. In January and February, and after these initial rainfall events, the amount of storm-water runoff was sufficient to increase the stream flow rate and result in reduced TOC concentrations. Unaccounted-for variation in model 3 may be due to unmeasured parameters, such as seasonal variations in coliform populations, varying sensitivities of coliforms to chlorine, and coliform masking in turbid samples. The statistical significance of the model may be evaluated on the basis of R^2, mean squared error, total squared error, standard error of the regression coefficient, and Student's t test values. Based on these criteria, the model was significant ($P < 0.01$) and unbiased (18).

CLDMD, which was important in predicting

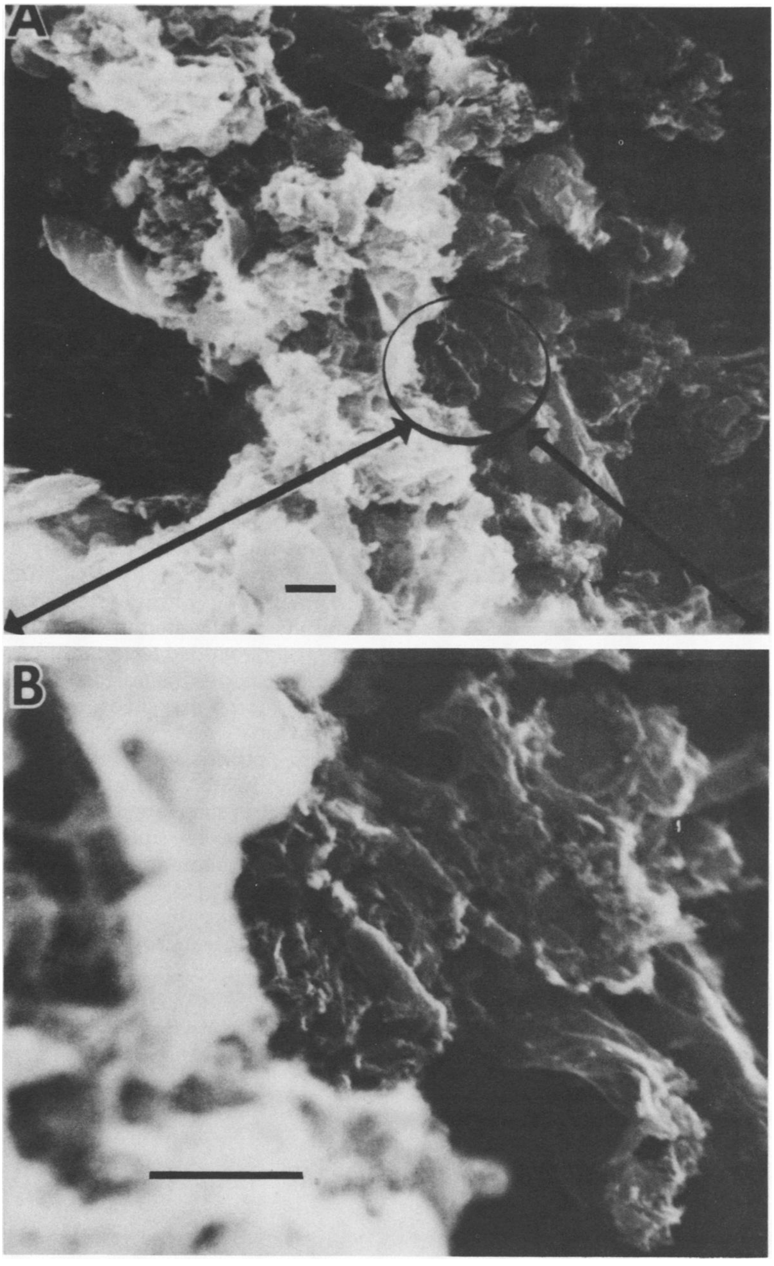

FIG. 3. *Scanning electron photomicrograph showing bacteria embedded in a particle. Bar, 1 μm.*

disinfection efficiency, was dependent upon the turbidity and the associated TOC content of water sample (Table 1). The turbidity and TOC variables explained 94% (R^2; Table 1) of the variation in CLDMD. Turbidity was found to be an accurate predictor of TOC levels in the raw water ($r = 0.83$; $P < 0.01$). These models were based on results obtained from six watersheds. In developing the three models, the data obtained from each of the watersheds were not found to be significantly different.

DISCUSSION

Turbidity may vary in its nature and composition from region to region, and since it is an optical property of a suspension, its measurement will be influenced by particle size, shape, number, and instrument characteristics (10, 12).

Despite these limitations, turbidity measurements in drinking water are valuable indicators of water quality because clay-organic complexes at entry points to unfiltered distribution water may act as carriers for a variety of materials such as pesticides, heavy metals, and bacteria (14, 16). Similar to the coliform indicator concept, a high turbidity measurement is usually an indication of inadequate water treatment. Turbidity determinations have advantages as indicators of water quality in that they are rapid and relatively inexpensive and can be performed continuously by in situ detectors.

The monitoring of coliform numbers during CLDMD determinations on water samples with elevated turbidities and statistical analysis of these results have shown that turbidity interferes with proper chlorine disinfection of drinking water. This conclusion applies to surface water sources which are not filtered before disinfection. In the Pacific Northwest region, numerous public water supplies use disinfection as the only treatment, even during periods of storm-water runoff. This results in elevated turbidity at both the raw water intake and at the consumer's tap. The disinfection efficiency model (model 3) predicts, assuming a constant chlorine dose, that an increase in turbidity from 1 NTU to 10 NTU in the surface water supply would result in an eightfold decrease in efficiency of disinfection. This could imply that turbid drinking water (10 NTU) is eight times more likely to carry pathogenic bacteria than water having a turbidity of 1 NTU. Though several studies have previously demonstrated that the presence of particulate material in water interferes with disinfection, those studies have dealt primarily with virus inactivation or coliforms within nematodes and crustaceans (2, 3, 17, 23). Since current practice utilizes coliforms as indicators of water potability, it is significant to note that naturally occurring turbidity at entry points to unfiltered distribution water interfered with disinfection of coliforms (Table 1). Our study included several different watersheds to demonstrate that results are probably applicable to most watersheds, at least in the Pacific Northwest.

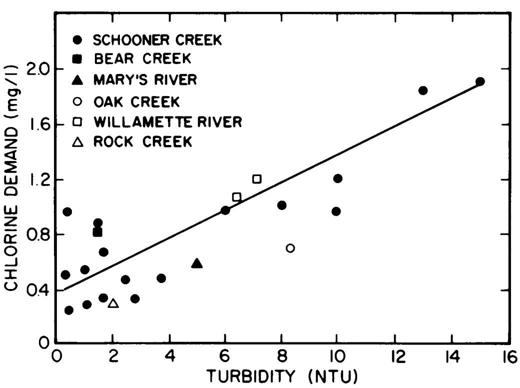

FIG. 4. *Relationship between turbidity and CLDMD in samples collected from six watersheds in western Oregon.*

A mechanism for coliform survival at high turbidity levels is possible if coliforms are embedded in suspended particles and chlorine is not able to come into contact with bacteria. Bacterial attachment to solids has been well documented (6, 14, 26, 27). The increase in bacterial numbers as a result of blending chlorinated samples and micrographs showing bacteria embedded in amorphous material provide further evidence that turbidity may protect bacteria from the action of chlorine.

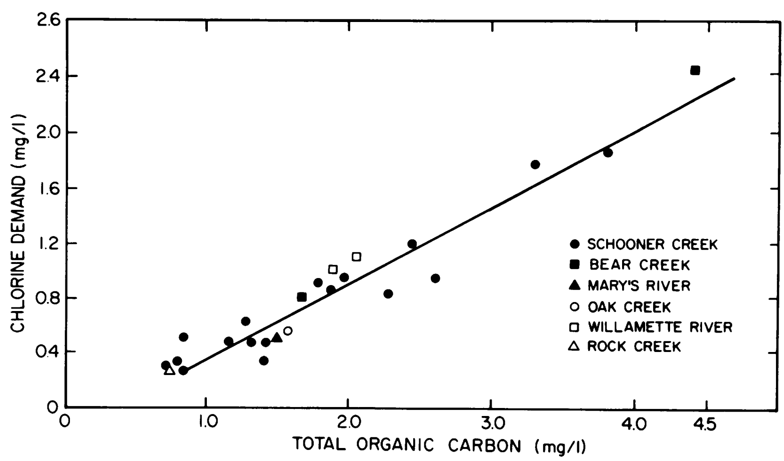

FIG. 5. *Relationship between CLDMD and TOC in turbid water samples collected from six watersheds in western Oregon.*

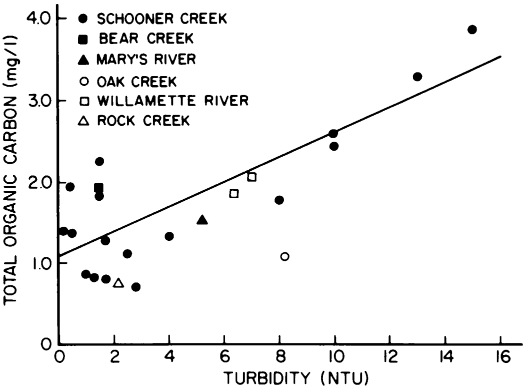

FIG. 6. *Relationship between TOC and turbidity in samples collected from six watersheds in western Oregon.*

TOC associated with turbidity was primarily responsible for causing CLDMD of surface waters. These results are consistent with what is known about chlorine chemistry. Organic compounds are known to react with hypochlorite to form trihalomethanes (28). The presence of high TOC levels in raw water supplies produces two undesirable effects; it reacts with chlorine, rendering it ineffective for disinfection, and the byproducts of this reaction are trihalomethanes, some of which are known to be carcinogenic (29).

The statistical models can be used to predict the impact of turbidity on drinking water quality, as the following illustrates. Consider a raw water sample containing 400 total coliforms per 100 ml, which is chlorinated with an initial dose of 1.0 mg of chlorine per liter (season = 12). If the turbidity of the raw water source is 1 NTU, a value of 1.2 mg of TOC per liter would be associated with the turbidity (model 1). With this TOC value, a CLDMD of 0.45 mg/liter could be predicted and could result in a concentration of 0.55 mg of free chlorine per liter being available as a chlorine residual in the distribution system (model 2). At 1 NTU, model 3 indicates that a 460-fold reduction in coliform numbers would result in disinfected drinking water containing less than 1 coliform per 100 ml. If the result of rainfall was that the raw water increased in turbidity from 1 to 5 NTU, then 1.83 mg of TOC per liter would be associated with the turbidity. This would result in a CLDMD of 0.81 mg/liter. If the chlorine dose remained constant, the residual after chlorination with 1.0 mg of chlorine per liter would be 0.19 mg of chlorine per liter, which is below the accepted limit for a desirable chlorine residual (24). A 180-fold reduction in coliform numbers would result in a reduction of the initial count of 400 coliforms per 100 ml in raw water to 2 coliforms per 100 ml in drinking water. This water would not meet the compliance regulation of not exceeding 1 coliform per 100 ml, as stated in the National Interim Primary Drinking Water Regulations (24).

The assessment of the bacterial quality of drinking water depends on the ability to make accurate microbiological determinations. The results indicated that turbidity interfered with the determination of coliform densities by the membrane filter technique. Both the incidence of masking and the magnitude of interference with coliform detection increased as turbidity increased. Fryt (7) has observed that with increasing turbidity values over 1.8 NTU, the differences between MF and MPN results tended to increase. Geldreich et al. (8) have noted that more coliforms could be detected in waters containing turbidities of 1 to 5 NTU than in any other range of turbidities. Our results indicated that the magnitude of coliform masking increased from <1 coliform per 100 ml in water samples of <5 NTU to >1 coliform per 100 ml in water samples of >5 NTU. In addition, coliforms could be recovered from 26% of 56 distribution samples that apparently lacked coliforms by the standard MF technique. Coliforms that were isolated most often from masked coliform samples were identified as *C. freundii* and *Enterobacter* spp. These organisms have been previously reported to be the predominant species in chlorinated drinking water (4).

Several explanations may account for failure to recover typical coliforms by the MF technique: (i) coliforms may be entrapped in suspended materials and may not be able to form colonies (8); (ii) high numbers of standard plate

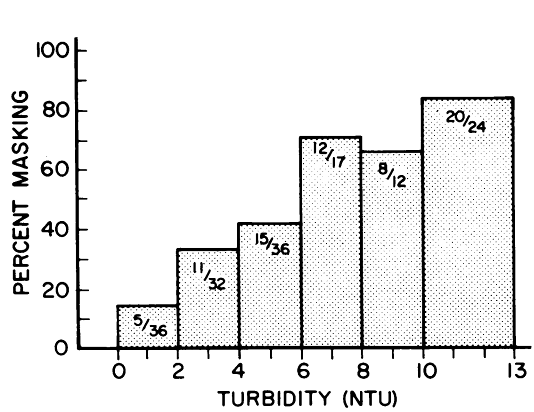

FIG. 7. *Relationship between percent of filters with masked coliforms and turbidity in chlorinated water (1.0 to 1.6 mg of free chlorine per liter). The number of filters with masked coliforms is presented over the total number of filters analyzed.*

TABLE 1. *Models derived to predict impact of turbidity on drinking water quality*

Model		R^{2a}	No. of observations	Squared error		SE of regression coefficients	*t* values
				Mean	Total		
(1) TOC[b] =	1.070	—[c]	23				
	+0.153 (NTU)						
(2) CLDMD[d] =	−0.075	0.936	17	0.0186	3.0	0.0989	−0.77
	+0.029 (NTU)					0.0413	2.02
	+0.405 (TOC)					0.0780	5.09
(3) DE[e] =	1.6951	0.663	32	0.1965	4.5	0.2448	6.92
	+0.0549 (Season)[f]					0.0257	2.13
	−0.1676 (NTU)					0.0325	−5.15
	+0.7763 (CLDMD)					0.3829	2.03
	+0.0003 (TC)[g]					0.0011	2.76

[a] R^2, Coefficient of multiple determination; SE, standard error.
[b] TOC in milligrams per liter.
[c] Multiple regression terms do not apply to linear regression involving one independent variable (18).
[d] CLDMD in milligrams per liter.
[e] DE, Disinfection efficiency measured as the $\log_{10}$ of the decrease in number of coliforms.
[f] A numerical term (1 to 12 for January [1] to December [12]) used to explain seasonal effects on disinfection efficiency.
[g] TC, Number of verified total coliforms.

count bacteria accompanying elevated turbidities may be antagonistic to coliforms (8, 9); or (iii) turbidity may alter the surface pore morphology of the membrane filters so that surface openings are not large enough to surround the entrapped bacteria, thus preventing optimum growth conditions (21). Coliform masking accompanying turbidity may be a combination of these factors.

The alternative technique to the MF procedure for detecting coliforms in drinking water is the MPN procedure. The MPN procedure also underestimates both the incidence and number of coliforms in drinking water samples (13). Based on over a year of field sample results, no relationship was found between the magnitude of coliform masking in the MPN and the turbidity of the water sample. Since both the MF and the MPN technique are subject to coliform masking, the presence or absence of coliforms in turbid samples (NTU >5) can best be determined with alternative techniques, such as the modified MPN (5).

In summary, turbidity is a useful indicator of potential problems in drinking water. Components of turbidity associated with TOC exert a continuous interference with disinfection efficiency, maintenance of a chlorine residual, and microbiological determinations. We can further conclude that turbidity control is justified to at least the 1- and 5-NTU levels to maintain adequate drinking water quality.

ACKNOWLEDGMENTS

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